Amendments to the Specification

Please replace the corresponding paragraphs of the Experimental of the application with the paragraphs shown below, in which amendments are marked, and which are preceded by the page and line at which they begin.

Page 20, line 26:

**1.1 Reagents**

The P-selectin Fc fusion protein (P-selectin.Fc) was obtained in lyophilized form from R&D Systems (Minneapolis, MN). Aliquots of P-selectin.Fc were made by dissolving 750 µg of the lyophilized protein in 15 µL of Dulbecco's Phosphate Buffered Solution (DPBS) (Invitrogen, UK), which were frozen in liquid nitrogen and stored at –20° C for less than 4 months. Prior to biotinylation, the anti-P-selectin antibody Rb40.34 stock was dialyzed in DPBS overnight using a 10,000 Dalton MWCO dialysis cartridge (Pierce, Rockford, IL). N-hydrosuccinimido-biotin (NHS-biotin) and streptavidin for the antibody-microbubble linkage system were obtained from Sigma (St. Louis, MO). Eu-labeled streptavidin and DELFIA solution were obtained from Wallac Oy (Turku, Finland). Advanced Protein Assay Reagent used in determining antibody concentrations was obtained from Cytoskeleton, Inc (Denver, CO). Blocker Casein in TBS used to block non-specific adhesion in flow chamber experiments was obtained from Pierce (Rockford, IL). ~~Tween-20~~ TWEEN-20 was obtained from J.T. Baker (Phillipsburg, NJ). ~~Isoton-II~~ ISOTON-II used as a diluent for the ~~Coulter~~ COULTER counter was obtained from Beckman Coulter (Miami, FL). Kimura

[0.05% wt/vol toluidine blue, 0.9% NaCl in 22% ethanol, 0.03% light-green SF yellowish, saturated saponin in 50% ethanol, and 0.07 M phosphate buffer, pH 6.4; all from Sigma (St. Louis, MO)] was used to stain leukocytes for blood counts (Olsen and et al, 2001).

Page 21, line 17:

**1.2 Inverted Parallel Plate Flow Chamber**

In vitro adhesion efficiency experiments were performed with a parallel plate flow chamber (GlycoTech, Rockville, MD). The distance between the top and bottom plates, determined by the gasket thickness, was 0.254 mm, and the flow path width was 2.5 cm (gasket "B"). 35 mm diameter polystyrene dishes (Corning, Corning, NY), on which the P-selectin.Fc substrate was plated, served as the top plate of the flow chamber. Because of the buoyancy of the microbubbles, the flow chamber was used inverted by means of a specially fabricated holder. The dish was secured onto the flow chamber by vacuum suction and circumferential rubber bands, and the entire flow chamber was inserted into the inverted holder. A syringe pump (Harvard Apparatus, Cambridge, MA) in the aspiration mode was used to draw the microbubble dispersion through the flow chamber at defined shear rates. Experiments were visualized with a ~~Leitz Laborlux~~ LEITZ LABORLUX II microscope (Rockleigh, NJ) using partial epifluorescence with a 40X objective (Olympus, Tokyo, Japan). Data from the flow chamber experiments was recorded on standard VHS cassettes (Sony, Tokyo, Japan) or digital video cassettes (Sony, Tokyo, Japan)

for subsequent off-line analysis. A diagram of the inverted parallel plate flow chamber is presented in Figure 1.

Page 23, line 26:

After two washes to remove small microbubbles and free and micellar lipids, the Di-I labeled microbubble population was partially crushed as follows. The 2.0 mL dispersion was drawn into a 10 mL syringe containing 8.0 mL of air. The syringe was closed and depressed such that the volume was decreased to 6.5 mL and 750 mmHg was distributed uniformly among all bubbles in the dispersion. Pressure applied to the dispersion was measured with an electronic manometer (Dwyer, Michigan City, IN). Repeated washings eliminated any empty shell fragments from the wrinkled population, and produced a more uniform size distribution for both populations, as measured by a ~~Coulter~~ COULTER counter (Beckman-Coulter, Miami, FL).

Page 24, line 15:

**1.7 P-selectin.Fc Site Density Determination**

Known concentrations (25, 150, or 250 ng in 200 µL) of P-selectin.Fc were adsorbed to 35 mm dishes as described above. Following 2 hrs of blocking in casein, 0.96 ug of biotinylated Rb40.34 in 1.0 mL DPBS was added to each dish. The amount of solvent was sufficient to cover the entire surface of the dish. After 30 minutes of incubation at room temperature, unbound antibody was removed by 7 washes with 0.05% ~~Tween-20~~ TWEEN-20, and 0.1 µg Eu-labeled

streptavidin in 1.0 mL was added to each dish. After 30 minutes of incubation at room temperature, the dish was washed 7 times to remove unbound streptavidin. 0.9 mL of DELFIA enhancement solution was added to each plate and incubated for 5 minutes at room temperature. The reactant was collected from each dish and placed in a 96-well microtitre plate (300 µL per well) for time-resolved spectrofluoroscopy using a ~~SPECTRAmax Gemini~~ SPECTRAMAX GEMINI XS dual-scanning microplate spectrofluorometer (Molecular Devices, Sunnyvale, CA). Plates were excited at 360 nm and read at 610 nm during a 250-1250 µs timer interval. Scanning plates incubated with casein alone determined the level of non-specific antibody adhesion. Rb40.34 biotinylation was determined to be approximately 0.3 mole biotin per mole antibody using an avidin-HABA displacement method (Green, 1965). Site densities of adsorbed P-selectin.Fc were calculated assuming 1-to-1 binding of Rb40.34 to each head of the P-selectin.Fc dimer, and 1-to-1 binding of streptavidin to Rb40.34.

Page 29, line 1:

**1.12 Statistical Analysis**

The attachment efficiency of spherical and wrinkled microbubbles was compared at each of the three applied wall shear rates for the flow chamber experiments. A paired t-test was performed using the ~~Excel~~ EXCEL v 9.0 spreadsheet package (Microsoft). Significance was tested at $p = 0.05$. The mean number of adherent microbubbles in the 10 examined venules was

compared in the in vivo experiments. The same paired-sample t-test was performed for these data.

Page 30, line 27:

The ~~Coulter~~ COULTER multisizer was used to obtain size characteristics of the two microbubble populations. The ~~Coulter~~ COULTER counter calculates the size of a particle by measuring its electrical conductivity. Thus, the size distributions obtained from the ~~Coulter~~ COULTER counter report the size of the gas core of each microbubble population, and do not account for the irregularities in the surface of the wrinkled bubbles. A difference in diameter of approximately 0.5 μm between the two microbubble populations was apparent from the measured size distributions (Figure 5). This size difference confirms that the wrinkled microbubbles have lost some of their gaseous volume, and thus converted some lipid surface area to flat, volumeless folds. The gaseous volume of a microbubble of either population may be calculated as the volume of a sphere of diameter equal to that reported by the Coulter counter. The volume of gas in an average wrinkled microbubble was 7.7 $\mu m^3$, while that of an average spherical microbubble was 13.2 $\mu m^3$. Hence, 5.6 $\mu m^3$ of $C_4F_{10}$ was excluded from an average wrinkled microbubble, which represents a volume loss of 41%.